Attorney's Docket No. 44158/209598(5853-3) PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Evans et al.	Confirmation No.:	2302
Appl. No.:	09/829,113	Group Art Unit:	1634
Filed:	April 9, 2001	Examiner:	Jeffrey N. Fredman
For:	HAPLOTYPING METHOD FOR MULTIPLE DISTAL NUCLEOTIDE POLYMORPHISMS		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RULE 37 C.F.R. § 1.132 DECLARATION
of Oliver Gene McDonald and William Evans

I, Oliver Gene McDonald and I, William Edward Evans, do hereby declare and say as follows:

1. I, Oliver Gene McDonald, am an inventor of the subject matter of the above-captioned application.

2. I, Oliver Gene McDonald, am skilled in the art of the field of the invention. I am pursuing a M.D./Ph.D. in molecular physiology from University of Virginia. I have a Bachelor of Science degree in biology from University of Tennessee. I have doctoral training from Dr. Gary K. Owens of University of Virginia. Since 2001, I have been engaged in the study of molecular biology, and particularly of genomic DNA. I have been employed by St. Jude from 2000-2002, and was in their Pharmacogenetics Group.

3. I, William Edward Evans, am an inventor of the subject matter of the above-captioned application.

4. I, William Edward Evans, am skilled in the art of the field of the invention. I have a Pharm.D. from University of Tennessee, Memphis. I have a Bachelor of Science degree in Pharmacology from University of Tennessee. I have doctoral degree in Clinical

Pharmacology from the University of Tennessee. Since 1987, I have been engaged in the study of molecular biology, and particularly of Pharmacogenetics and Pharmacogenomics. I have been employed by St. Jude since 1976, and have been the Scientific Director since 2001.

5. I have read and understood the Office Actions in the above case dated 12 December 2002, 7 July 2003, and 24 November 2001. I have also read and understood references cited and discussed in this case, including Li *et al.* (1998) *BioTechniques* 25:358-361, Patel *et al.* (1991) *Nucleic Acids Res.*, 19:3561-3567, and Michalatos-Beloin *et al.* (1996) *Nucleic Acids Res.* 24:4841-4843.

6. The method of Patel *et al.* (1991) *Nucleic Acids Res.*, 19:3561-3567 is conceptually and technically different from our method. These differences include the fact that our claimed method isolates the target sequence from genomic DNA before circularization (ligation) by amplifying a DNA fragment by PCR. In contrast, the Patel *et al.* method isolates the target sequence after circularization of total genomic DNA. Further, our claimed method involves isolation of target sequence from undigested and unligated genomic DNA by PCR techniques, including long range PCR. In contrast, Patel *et al.* isolates target sequence via short-range Allele-specific PCR from digested and ligated genomic DNA and production of a hemizygous target sequence. See the final slide of our Communication filed February 11, 2003 (hereinafter "Slide 4," provided for the reader's convenience as Appendix A of the response filed concurrently herewith).

7. The method of Michalatos-Beloin *et al.* (1996) *Nucleic Acids Res.* 24:4841-4843 is conceptually and technically different from our method. These differences include the fact that our initial isolation of the target sequence produces heterozygous target sequence, whereas Michalatos-Beloin *et al.* produces hemizygous DNA. Thus, we are not limited to haplotype analysis via isolation of hemizygous DNA. Michalatos-Beloin *et al.* themselves emphasized that their method produces hemizygous DNA by stating at page 4867, column 2, that "the ability to isolate hemizygous DNA segments from heterozygous genomes via molecular haplotyping will provide the accuracy necessary in these diverse applications." See the third slide of our

Communication filed February 11, 2003 (hereinafter "Slide 3," provided for the reader's convenience as Appendix A of the response filed concurrently herewith).

8. The method of the newly cited reference to Li *et al.* is also conceptually different from our claimed method. In my scientific opinion, Li *et al.* is distinguishable from our method on grounds including the following points.

- Li *et al.* does not circularize in a way that brings polymorphisms closer together the way our claimed method does. Figure 1 of our application and Figure 1 of Li *et al.* clearly demonstrate this result. When in linear genomic DNA, the distal-most polymorphisms, M-G/T in intron 1 and M(G/T)-N(AG) in exon 2, are separated by 30-43 base pairs (page 358, second column, line 12). Once circularized by Li *et al.*, the polymorphisms are the *same* distance from each other in one direction of the circle and *farther apart* in the other direction (a 357 base pairs linear molecule was circularized, thus 357 minus 30-43 gives the polymorphisms a distance of between 314 to 327 base pairs from each other).

- Li *et al.* does not provide the advantages of our method. By using inverse PCR, Li *et al.* simply generate back the same, or nearly the same, linear molecule that was circularized by using inverse PCR (after inverse PCR off of a 357 base pairs circle, Li *et al.* generate a 351 and a 366 base pairs fragment). In my scientific opinion, had Li *et al.* intended haplotype analysis of circular molecules the way our method allows (i.e. aimed at analyzing polymorphisms that had been brought into closer proximity), then Li *et al.* would *not* have used inverse PCR because inverse PCR simply generates back the same, or nearly the same, linear molecule that was circularized in the first place.

Because of these differences, one would not combine Li *et al.*'s method with Patel *et al.* or Michalatos-Beloin *et al.* Further, because of these differences, the combination of these dissimilar methods would not result in a workable method.

9. Li *et al.* concludes with the following statement: "Although these alleles can be typed by allele-specific nested PCR following GPA-specific PCR, ASIP [allele specific inverse PCR], rather then nested PCR, can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR." Li *et al.*, page 361, column 1. When the statement of Li *et al.* is placed in context, it is clear that Li *et al.* refers to haplotyping polymorphisms that are too distantly separated for the design of a single forward PCR reaction. To place the comment in context, one must consider the following two points: (1) The only problematic distance discussed by Li *et al.* is a separation of 30-40 base pairs which causes a primer design problem for forward allele-specific PCR, necessitating the use of allele-specific nested PCR. Li *et al.*, page 358, column 2. (2) Li *et al.* solves the problem by use of a circularization method that does not bring the polymorphisms closer (see paragraph 8, above) followed by inverse PCR. Conversely, Li *et al.* circularizes for the specific purpose of making the polymorphisms further apart so that AISP can be used rather than nested PCR. This is because the polymorphisms are too far apart for a single allele-specific primer to be designed for amplification, and too close together (30-43base pairs) for a single nested PCR amplifications to produce a band that could be visualized on standard agarose/ethidium bromide gels (it is common knowledge that it is very difficult to visualize bands less than 100 base pairs on these gels). By making the polymorphisms further apart, a single ASIP reaction can be performed that produces products that are long enough (351 base pairs and 366 base pairs) to be visualized on agarose gels. This is demonstrated by the fact that Li *et al.* circularizes to make the polymorphisms further apart in one direction, followed by inverse PCR that amplifies products of 351 base pairs and 366 base pairs which are much longer than the 30-43 base pairs that separates C/T and G/T. If Li *et al.* had intended to bring polymorphisms closer together, they would have done so by circularizing to bring the polymorphisms closer together for a single PCR primer to be designed, and they would have then used a forward, not an inverse PCR, off of the circularized template. In contrast, Applicants' method is designed to haplotype polymorphisms that are distantly located in *genomic* DNA by bringing the polymorphisms into closer proximity via circularization.

10. In greater detail, Li *et al.* faced several problems when haplotyping the MN blood group system (the subject of their article). First, the polymorphisms Li *et al.* utilized to type the M^G, M^T, and N alleles of the GPA gene also occurred in the GPB and GPE genes, which are over 95% homologous with the GPA gene. Li *et al.* solved this by carrying out GPA-specific PCR. Li *et al.*, page 358, column 2. Second, the M^G, M^T and N alleles cannot be typed by the single allele-specific PCR amplification technique. Li *et al.*, page 358, column 1. Li *et al.* explains the problem as follows.

> Although primers specific to M^G and N alleles can be designed, the M^T- (and N-) specific nucleotide T in intron 1 is located *too far* (30-40 bp upstream) from the three M- (M^G and M^T) specific bases in exon 2 to design a single M^T-specific primer.

Li *et al.*, page 358, columns 1-2 (emphasis added). The inability to design a single M^T-specific primer can be overcome by allele-specific nested PCR after GPA-specific PCR. Li *et al.*, page 361, column 1. However, Li *et al.* chose to use circularization and one round of ASIP, which allows for a *single* procedure using allele-specific primers. Li *et al.*, sentence spanning pages 360-1. Li *et al.* correctly notes on page 361 that multiple allele-specific PCRs (nested necessarily refers to multiple) can be performed for haplotpe analysis after GPA-specific amplification, so the statement on page 361 refers back to the problem on page 358, in that "AISP [a single procedure]...can be applied to haplotyping polymorphisms separated by a distance that is too far to be amplified by PCR." It is my scientific opinion that this sentence refers to the fact that the polymorphisms are separated by a distance too great for a *single* primer to be designed that could anneal to a region that contains multiple polymorphisms (in intron1 and exon2 here) for a *single* allele-specific PCR procedure (and thus requiring either nested/multiple PCRs or Li *et al.*'s single AISP method). PCR here means a single PCR reaction, rather than if they had said nested PCR, which requires multiple PCR reactions. Thus, the problem of distance Li *et al.* refers to is polymorphisms being too far apart to design a primer for a single PCR procedure, and not a problem of polymorphisms being too far apart in genomic DNA for conventional haplotype analysis, as Li *et al.* notes that these polymorphisms can be haplotyped by RFLP and SSCP. Li *et al.* recognizes the following three problems of distance:

1. Too far for primer design for a single PCR reaction
2. Too close for visualizing short products of allele-specific PCRs on a gel
3. Having to perform multiple nested PCRs to overcome these 2 distance problems

Li *et al.* circumvents the need for multiple nested PCRs by circularizing to make the polymorphisms farther apart, followed by a single ASIP procedure that does not require a primer to anneal to a region containing multiple polymorphisms and produces products that are long enough to clearly see on a gel.

11. It is my scientific opinion that Li *et al.* performs circularization and ASIP to circumvent the need for allele-specific nested PCRs from genomic DNA, not to bring polymorphisms closer together. In other words, the last statement by Li *et al.* means that ASIP should be used in place of PCR, not combined with PCR.

12. It is my scientific opinion that the combination of the methods of Li *et al.* and Michalatos-Beloin *et al.* does not produce our claimed method. If one were to generate hemizygous templates with Michalatos-Beloin, then perform the circularization and ASIP of Li, it would not generate a shorter fragment with the polymorphisms closer together because (1) the circularization step does not bring the polymorphisms closer and (2) Li *et al.'s* PCR primers for the ASIP are in opposite orientation from ours (compare Li, Figure 1 with Figure 1 of our application). This in itself is conceptually *different* from our method, as we do not perform inverse PCR as exemplified by Li *et al.* from circularized templates. It is impossible to generate the fragments for haplotype analysis that our method does by using inverse PCR off of circular molecules.

13. In summary, the combination of Li *et al.* with Michalatos-Beloin *et al.* results in allele-specific amplification producing hemizygous target, followed by circularization and allele-specific inverse PCR, which simply regenerates the same or similar linear fragment that was first amplified by the Michalatos-Beloin isolation method. Notably, Li's method of circularization does not bring the nucleotide polymorphisms closer together. Patel *et al.* cannot be combined

with these other references to produce our method because Patel *et al.* teaches circularization of genomic DNA before isolation of the target (unlike Michalatos-Beloin *et al.* and Li *et al.*, which both isolate the target first).

14. For the above reasons, based on my education and scientific experience, I believe that one working in this field would not arrive at our method given the references by Li *et al.*, Patel *et al.*, and Michalatos-Beloin *et al.* Based on my education and scientific experience, I further believe that one working in this field would not be motivated to combine or modify these references based on the references themselves or the state-of-art.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Oliver Gene McDonald — Date: 3-11-04

William Edward Evans — Date: 3-17-04